AN INVESTIGATION INTO THE DISTRIBUTION OF AUTOTROPHIC AND HETEROTROPHIC BACTERIA IN LITTLE ROUND LAKE, WITH PARTICULAR EMPHASIS ON SOME BACTERIAL AGENTS OF THE SURFACE CYCLE

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AN INVESTIGATION INTO THE DISTRIBUTION OF AUTOTROPHIC AND HETEROTROPHIC BACTERIA IN LITTLE ROUND LAKE, WITH PARTICULAR EMPHASIS ON SOME BACTERIAL AGENTS OF THE SULFUR CYCLE

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December, 1969

TABLE OF CONTENTS

- Introduction -

In certain waters having an anoxic environment, e.g. meromictic lakes, hydrogen sulfide can be detected in the hypolimnion throughout the year. The importance of microorganisms in the metabolisms of sulfur compounds in these lacustrine environments cannot be overlooked. The study of the rate and mechanism of H_2S oxidation and sulfate reduction is important in the processes of energy transformations, bacterial biosynthesis and in considering the oxygen regime of a body of water.

In the sulfur cycle, photosynthetic green and purple sulfur bacteria and chemosynthetic thiobacteria are implicated in primary production, while the activities of anaerobic sulfate-reducing bacteria may produce the H_2S substrate for growth of the former types.

Kuznetzov (5) has described the role of bacteria involved in the sulfur cycle in lakes. Hydrogen sulfide may be generated by the anaerobic bacterial reduction of sulfate or by the decomposition of protein and organic sulfur compounds. The oxidation of reduced sulfur can proceed under both anaerobic and aerobic conditions. Pigmented photosynthetic sulfur bacteria may bring about the oxidation of sulfide to elemental sulfur or sulfate. Under microaerophilic or aerobic conditions, the oxidation of sulfides to molecular sulfur or sulfate is brought about by the colorless thionic bacteria (Thiobacillus thioparus and T. thiooxidans). In reservoirs, the sulfur-oxidizing microorganisms

...2

showed the highest activity at the interface between the H_2S and oxygenated layers of water (5). In addition to bacterial participation, H_2S may be oxidized chemically in the water column (13).

Sorokin (13,14) has presented evidence to show that chemosynthesis by bacterial oxidation of sulfides is one of the intrinsic sources of primary production in meromictic lakes.

Barvenik and Jones (1) reported that chemosynthetic productivity was of significance in the ecology of the sulfur cycle in an eutrophic coastal pond near Wood's Hole, Mass.

Takahashi and Ichimura (15) found dense populations of certain members of the Chlorobacteriaceae and Thiorhodaceae in the upper layer of the H_2S zone in a Japanese meromictic lake. The organic matter synthesized by these photosynthetic sulfur bacteria ranged from 9% to 25% of the total annual primary production in lakes rich in H_2S. Purple sulfur bacteria (Chromatium) have also been found abundantly in the chemocline of meromictic Green Lake (2).

In studies of Lake Gek-Gel, Sorokin (14) obtained data to show that the magnitude of chemosynthesis was commensurate with the extent of photosynthesis in this meromictic lake. No photosynthetic bacteria were found, since no light penetrates to the H_2S zone which begins at a depth of 28 m. In the 28-35 m layer, active oxidation of reduced sulfur by thionic bacteria occurred. No thiobacterial populations were observed in the water column above and below this zone. In this chemosynthetic zone, only about 15% of the total bacterial population

...3

were heterotrophic, the remaining 85% possessing an autotrophic nature. Autotrophic nitrifying bacteria accounted for significant chemosynthesis in the oxygenated surface layer of Lake Gek-Gel.

In meromictic Lake Belovod, the H_2S zone begins at a depth of 10 m. Photosynthetic sulfur bacteria occurred at the chemocline (10-14 m). Maximum chemosynthesis by autotrophic thionic bacteria occurred in a zone from 8-14 m (13).

The anaerobic sulfate-reducing bacteria of the genus Desulfovibrio play an important role in the sulfur cycle, using sulphate as a terminal election acceptor for their respiration, generating H_2S in the process. Postgate (10) had discussed these unique microbes in a review article.

It has been reported that in Lake Balkhash, U.S.S.R., the numbers of sulfate-reducing bacteria were relatively small in the water, with the bulk concentrated in the sediments. Numbers reached tens and hundreds of thousands per gram sediment and were higher during the summer than in winter. The intensity of the sulfate reduction process was apparently related to the concentration of sulfate and available organic matter in the muds.

Chemosynthetic sulfur oxidizing bacteria were also found in large quantities in the sediment (up to 500,000 per g). The H_2S produced in muds of this lake was not accumulated in large quantities, because it was oxidized by chemosynthetic and photosynthetic sulfur bacteria (6).

...4

Sorokin (14) found that sulphates were reduced at the greatest rates in a zone just below the zone of intense chemosynthesis (35 m) and at the bottom of Lake Gek-Gel. Sulfate-reducing activity paralleled the abundance of sulfate-reducing bacteria. The sources which give rise to the intense reduction of sulfates in this meromictic lake are allochthonous organics which enter from run-off water and organic matter produced in the lake. The involvement of allochthonous organics via the sulfur cycle in the cycle of matter explains the basic peculiarity of the lake's regime. Although the lake is classed as oligotrophic, the distribution of H_2S and O_2 in the lake are characteristic of an eutrophic body of water.

It has been reported that in Lake Mendota, nearly 45% of the sulfide-sulfur in the sediment was derived from the mineralization of organic matter with the remaining 55% coming from the bacterial reduction of sulfate (7).

Little Round Lake is a meromictic lake, situated in Frontenac county, Ontario, near the intersection of highways 7 and 509, approximately 2 miles north of the village of Sharbot Lake. The lake has a surface area of 17 acres and a total depth of 16 m with a flat basin and fairly steep sides. The mixolimnion extends from the surface to a depth of 10 m, where the dissolved oxygen level drops off to 0 % saturation. No mixing occurs in the monimolimnion extending from 10-16 m. The H_2S zone reaches from 11 m to the bottom. The H_2S concentration increases gradually to 10 ppm at 13 m and reaches

a maximum of 20 ppm at 16 m near the bottom. The sulfate concentration in the water column is about 20 ppm at the surface, reaches a maximum of 40 ppm at 11 m, then drops to 10 ppm at 15 m.

In the monimolimnion, the concentration of total dissolved solids is much higher than in the zone above 10 m, with ammonia occurring at a rather high concentration and nitrate very low (8).

Certain members of the Athiorhodaceae and Chlorobacteriaceae which have been isolated from Little Round Lake occurred most abundantly in the chemocline zone near 11-12 m (8). Moreover, Brown (3) has reported on the presence and characteristics of carotenoids derived from the Athiorhodaceae in the sediments of Little Round Lake.

The purpose of this bacteriological investigation was to determine the distribution of thiobacteria, sulfate-reducing and heterotrophic bacteria in the water column and sediment of Little Round Lake in relation to depth and season, with the hope that the findings might lend some support to the intensive lake ecological study being conducted by Dr. R.S. Brown and R. McNeely of Queen's University. Because sulfur is an important element in meromictic lakes where it indicates redox processes and contributes to primary production, the study of the bacteria involved in the sulfur cycle in these environments, should further our knowledge of the significance of these specialized microorganisms in the ecosystem.

...6

PROCEDURES:

Water and sediment samples from Little Round Lake were taken periodically from November, 1968 to October, 1969. Samples were refrigerated and shipped within 24 hours of the time of sampling to the OWRC Toronto lab for determination of the following bacteriological parameters: sulfate-reducing bacteria (Desulfovibrio), autotrophic sulfur-oxidizing bacteria (Thiobacillus thioparus and T. thiooxidans). Samples taken in October, 1969 were analyzed for autotrophic ammonium-oxidizing bacteria (Nitrosomonas sp.) and aerobic and anaerobic heterotrophic bacteria.

A membrane filtration method was used for the enumeration of Desulfovibrio sp. in water and sediment samples. The technique was essentially a modification of the submerged membrane plate method described by Tsuneishi and Goetz (18).

The medium used for the MF count of Desulfovibrio was based on modifying the media described by Tsuneishi and Goetz (18) and Postgate (9). This medium used to support growth of the sulfate-reducers had the following composition: K_2HPO_4, 0.6 g; $MgSO_4.7H_2O$, 0.5 g; NH_4Cl, 1.0 g; $CaCl_2$, 0.05 g; Na_2SO_4, 1.0 g; sodium lactate, 10.0 g; yeast extract, 1.5 g; Fe $(NH_4)_2$, $(SO_4)_2$, 0.1 g; sodium thioglycollate, 0.3g; agar, 10.0 g; distilled water, 1000 ml; pH 7.2.

Stock solutions of ferrous ammonium sulfate (1%) and sodium thioglycollate (1.5%) previously sterilized by membrane filtration were added (1 ml each per 100 ml agar) to the molten agar just before pouring the plates.

...7

A thin layer of the agar medium for sulfate-reducers was poured on the bottom of small 'Millipore' petri dishes and allowed to set. After filtration of the sample dilution, the appropriate membrane was placed with its surface facing downward on the agar. A thick layer of the agar medium was poured over the inverted membrane to a level near the upper rim of the plate. The plates were incubated anaerobically in Brewer jars using the H_2-CO_2 "Gas Pak" system (B.B.L.) at 25 °C for 10 days.

Growth of sulfate-reducing bacteria was indicated by the formation of (black)FeS in the environment of each colony. The development of colonies was apparently very slow and sometimes re-incubation of the plates for an additional week was necessary. Many colonies appeared as small black spots (< 1 mm diameter) on the membrane or as intense dark zones sometimes spreading across much of the plate area.

Some of the black colonies were picked from the membrane and transferred to Starkey's broth for sulfate-reducers [J.A.W.W.A. 40:1291 (1948)] . These bacteria which grew on Starkey's broth and reduced sulfate were gram negative, very motile curved rods and were characteristic of Desulfovibrio sp.

A most probable number (MPN) method was employed for the enumeration of the chemoautotrophic thiobacilli and nitrifying bacteria in the samples. Thiosulfate broth media used for the cultivation of the thiobacteria were essentially those described by Postgate (11) for Thiobacillus thiooxidans and T. thioparus.

...8

The ammonia broth medium for growth of Nitrosomonas sp. consisted of: $(NH_4)_2 SO_4$, 2.0 g; K_2HPO_4, 2.0 g; NaCl, 1.0 g; $MgSO_4.7H_2O$, 1.0 g; $CaCl_2$, 0.2 g; trace element solution,*1 ml; distilled water, 1000 ml; pH 7.4.

In the MPN procedure, a 3 test tube series of each medium previously sterilized by autoclaving was inoculated with aliquots of each sample or dilutions therefrom. All tubes were incubated aerobically in the dark for 4 weeks at room temperature (20° - 25°C).

At the end of the incubation period, all MPN tubes were checked for presence or absence of bacterial growth.

A diphenylamine - H_2SO_4 spot plate test was carried out on the series of Nitrosomonas broth tubes to check for evidence of ammonia oxidation. The procedure for the spot test for nitrite + nitrate was as follows:

(1) add 1 drop test solution in a depression of a spot plate.

(2) add 1 drop diphenylamine reagent** and mix.

(3) add 2 drops concentrated H_2SO_4.

A deep blue color indicates the positive presence of nitrite or nitrate.

* Trace element stock solution: manganese chloride, 0.8 g; zinc sulfate, 0.42 g; copper sulfate, 0.11 g; ferrous sulfate 2.0 g; molybdic acid, 0.03 g; cobalt chloride, 0.09 g; distilled water, 1000 ml; pH, 2.5.

** Diphenylamine reagent: a) Dissolve 0.7 g diphenylamine in a mixture of 60 ml concentrated H_2SO_4 and 28.8 ml distilled water;
b) cool the mixture and add slowly 11.3 ml concentrated HCl and let stand overnight before using.

...9

Determination of the growth of the thiobacteria was made by testing pH and sulfate concentration of the contents of the inoculated and incubated test tubes and comparing the results with those determined for sterile control media. Although tubes of sterile thiosulfate broth remained clear after incubation, a cream colored precipitate of elemental sulfur appeared in some inoculated tubes, apparently a product of thiosulfate oxidation.

A drop of thiosulfate broth from each inoculated tube in the MPN series was placed on a small segment of pH indicator paper (pH range 2-10). If the pH of the broth in the test tube had dropped appreciably from that of the sterile control tubes, a positive reading was designated for acid production in that tube.

An approximate determination for sulfate production in each tube of thiosulfate broth was made by adding 1 ml of the test broth to a large test tube containing 18 ml of distilled water. One ml of a 2% $BaCl_2$ solution was then added to each large tube and the contents shaken. Control tubes of sterile T. thiooxidans and T. thioparus media were treated in the same way. Turbidity in each tube after $BaCl_2$ addition could readily be measured by a visual comparison with the control. A definite milky turbidity of $BaSO_4$ appeared in positive tubes which was discernibly greater than the faint haze which developed in the control and negative tubes.

...10

Estimation of the numbers of autotrophic bacteria of each type was made by recording the number of positive determinations in each test for the MPN series and figuring the count from a standard MPN table.

In October, 1969, water and sediment samples were analyzed for some heterotrophic bacteria, using a variety of media and incubation temperatures. Water samples were obtained from the surface and at depths of 5, 10, 12 and 14 meters in the lake.

Counts of aerobic heterotrophic bacteria were determined by using nutrient broth or agar. Water samples were analyzed by the membrane filtration method, while a pour plate method using nutrient agar was used for dilutions of sediment samples. Replicate plates of each sample were incubated at three different temperatures, 10 °C for 12 day, 25 °C for 3 days and 37 °C for 2 days.

Counts of anaerobic heterotrophs (clostridia) were determined using two different media. SPS agar (Baltimore Biol. Labs.) was employed to detect numbers of H_2S-producing or sulfite-reducing clostridia and RCM or Reinforced Clostridial medium (Oxoid - British Drug House) to determine "total" numbers of clostridia.

The MF technique was used for water samples; the filter membrane was inverted and inserted in molten but cool agar media (SPS or RCM) in small (2" diameter) glass petri plates. Pour plates were made in SPS and RCM agar from the sediment sample.

...11

Replicate plates of SPS media from each sample were incubated anaerobically at 25 °C for 6 days and at 37 °C for 4 days. Plates of RCM from each water sample were incubated at 25 °C for 6 days and from the sediment sample at 25 °C for 6 days and 10 °C for 14 days.

...12

TABLE I: - Counts of Sulfate-Reducing and Sulfur-Oxidizing Bacteria in Water and Sediment Samples from Little Round Lake

Depth (m) — Count per 100 ml water or per gram dry wt. sediment.

	Desulfovibrio sp.				Thiobacillus thiooxidans				T. thioparus			
	Nov. '68	Mar. '69	July '69	Oct. '69	Nov. '68	Mar. '69	July '69	Oct. '69	Nov. '68	Mar. '69	July '69	Oct. '69
0	10	-	<10	<10	90	-	<3	9	-	-	43	1100
2	<10	25	10	<10	750	<3	4	93	-	240	460	4600
4	50	36	<10	<10	70	<3	<3	28	-	150	240	2100
6	-	<3	20	-	-	<3	9	-	-	210	93	-
8	<10	<3	10	<10	150	<3	15	4	-	<3	150	240
10	50	<3	10	30	280	<3	11	23	-	<3	150	460
12	<10	<3	20	-	90	<3	21	-	-	7	2600	-
14	<10	<3	20	90	90	<3	15	43	-	9	93	150
15	<10	25	-	-	<30	<3	-	-	-	<3	-	-
sediment	250	2100	-	290	-	<100	-	480	-	1400	-	3400

...13

TABLE II: - Counts of Aerobic and Anaerobic Heterotrophic Bacteria in Little Round Lake, Ontario (October, 1969)

Count per 100 ml water or per gram dry wt. sediment.

	Nitrosomonas	Aerobic count (nutrient agar)			Anaerobes (Clostridia)		
					SPS medium		RCM medium
Incub. temp.	25 °C	10 °C	25 °C	37 °C	25 °C	37 °C	25 °C
Depth (m)							
0	<3	4700	6300	11,000	26	36	1400
5	<3	1500	500	2,800	52	28	1200
10	<3	160	500	310	16	36	1700
12	<3	210	300	150	130	20	210
14	<3	140	420	210	44	25	120
sediment	<100	43,000	23,000	12,000	32	700	5,000

...14

RESULTS AND DISCUSSION:

The raw bacteriological data obtained from the investigations in November, 1968, and March, July and October, 1969, is presented in Tables I and II.

The seasonal distribution of Thiobacillus thiooxidans and T. thioparus in the water column of Little Round Lake is graphically illustrated in Figs. 1(a) and 1(b) respectively.

In March, 1969, T. thiooxidans was not detected in the water column. In July, numbers were very low, ranging from <3/100 ml at the surface to a maximum of only 21 at 12 m, while in October, increased populations were observed at depths of 2 m (93/100 ml) and 14 m (43/100 ml). In November, 1968, highest numbers of T. thiooxidans were found with maximum counts of 750 and 280 per 100 ml occurring at depths of 2 and 10 m respectively. At all other depths, numbers approximated 100.

The population of T. thioparus in samples taken in March was the lowest observed for all seasons (Fig. 1(b). Numbers were somewhat higher in the mixolimnion (approx. 200/100 ml) than in the H_2S zone (<3/100 ml).

In July, the population of T. thioparus showed a slight increase over that observed in March. Maximum numbers were observed at 2 m (460) and 12 m (2600/100 ml). Greatest numbers of these thionic bacteria were observed in samples taken in the October survey, with a maximum population of 4600 occurring at 2 m. Numbers declined to

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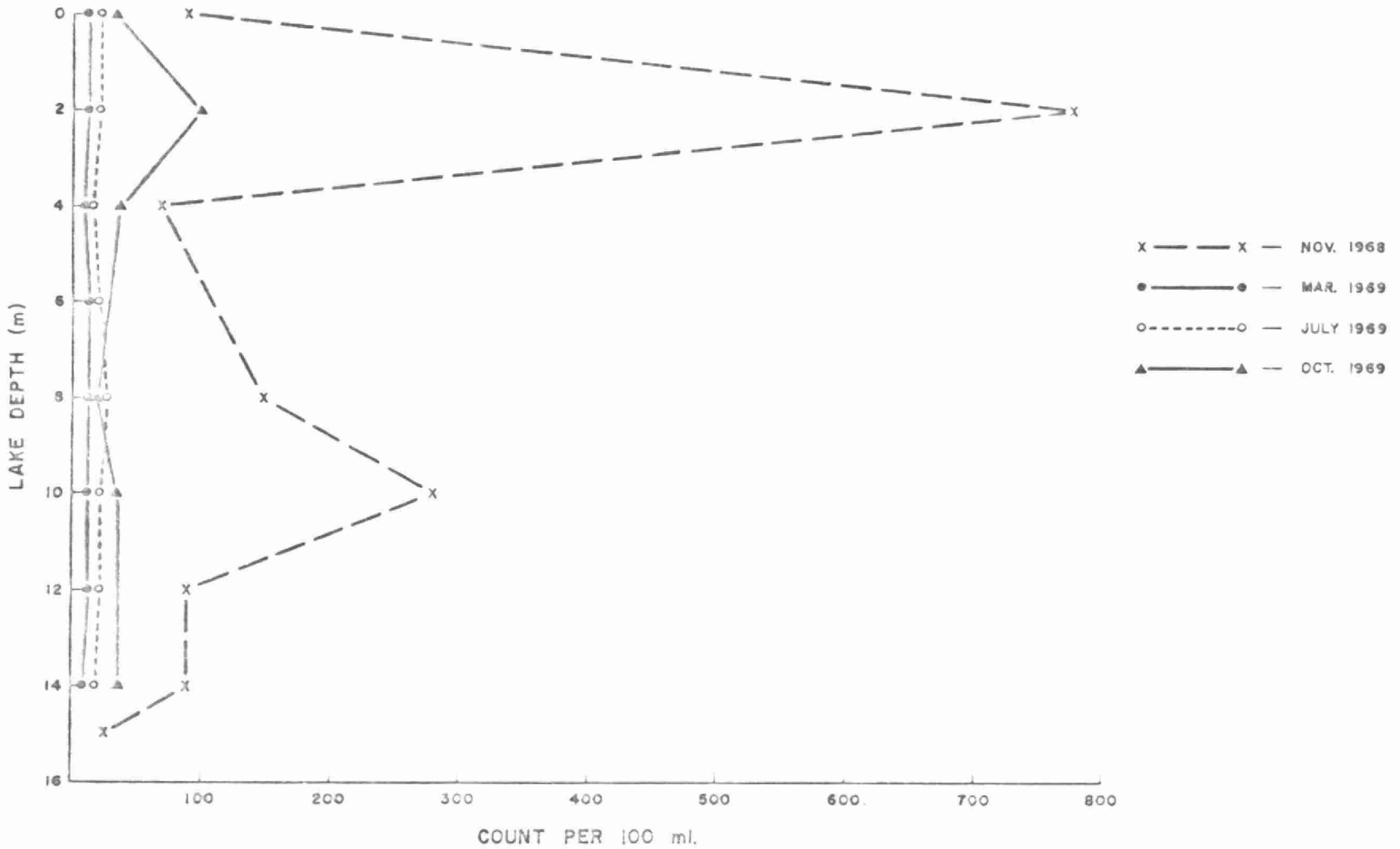

FIG. 1 (A) – SEASONAL DISTRIBUTION OF T. THIOOXIDANS IN WATER COLUMN OF LITTLE ROUND LAKE

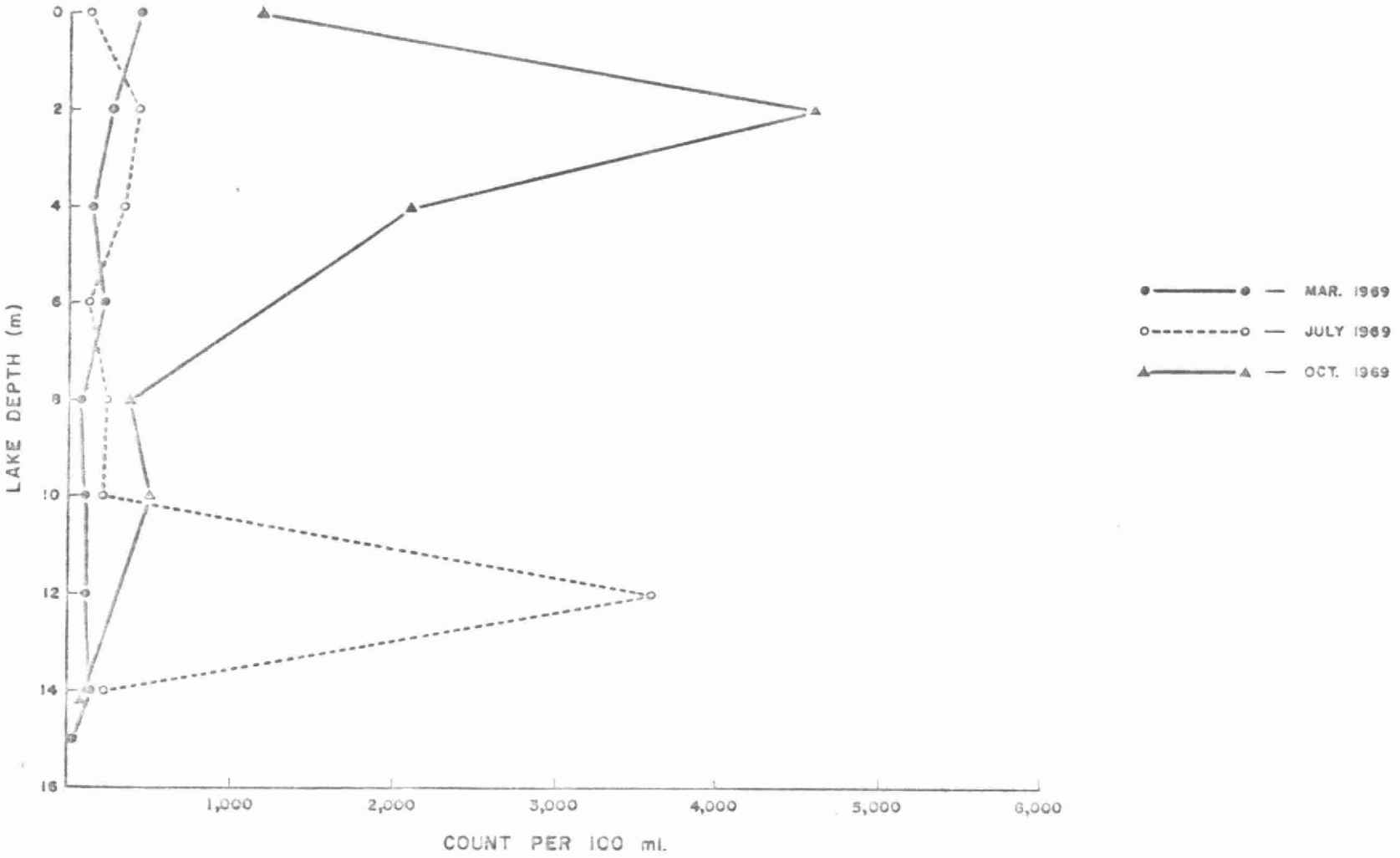

FIG. 1 (B) – SEASONAL DISTRIBUTION OF THIOBACILLUS THIOPARUS IN THE WATER COLUMN OF LITTLE ROUND LAKE

240/100 ml at 8 m, increased to 480 at 10 m and declined again at 14 m to 150. Unfortunately, no sample was analyzed from 12 m, so we could not determine if a maximum population occurred at this depth in October as happened in July.

Results obtained by Barvenik and Jones (1) indicated that a peak in numbers of thiobacilli occurred at the same depth as maximal chemosynthesis. Up to 2400 T. thioparus cells/100 ml were detected with lower numbers of T. thiooxidans and T. denitrificans occurring in the eutrophic coastal pond.

In Little Round Lake, populations of thionic bacteria in the water column were highest in autumn, and lowest in March, with maximal levels occurring at the 2 m depth in the mixolimnion and at the 10-12 m depth in the upper layer of the H_2S zone.

In both water and sediment samples, counts of T. thioparus were about 100 times greater than numbers of T. thiooxidans, suggesting that the former were a more active and significant group than the acidophilic T. thiooxidans in the lake environment.

The peak population of T. thioparus which was observed at 12 m in July may have been attributed to improved growth conditions for the bacteria at this depth. Thermal conditions were improved from March and available reduced sulfur substrate was abundant at this depth where a bloom of purple photosynthetic bacteria occurred during the summer. Microaerophilic conditions to support growth of the sulfur oxidizing bacteria could possibly have prevailed in the

...18

upper H_2S zone near 11 m where sulfate, the product of sulfur metabolism was found at maximal concentration in the water column.

The observed increase in numbers of thiobacteria at 2 m in the summer and autumn, although remarkable, cannot yet be fully explained. Perhaps H_2S resulting from the decomposition of phytoplankton and algae at the 2 m zone stimulated growth of the thiobacilli in this environment.

Sulfate-reducing bacteria were practically undetectable in the water column at all seasons. All counts of Desulfovibrio varied from <10 - 50 per 100 ml and no significant differences with respect to vertical or seasonal distribution in the water column were noted. The sulfate-reducers would not normally grow in the oxygenated mixolimnetic water, but in the H_2S zone where the Eh is poised sufficiently low for growth to occur, other factors, e.g. temperature, organic matter and nutritional or toxic conditions may have been limiting to bacterial growth.

The population distribution of the thiobacilli and sulfate-reducing bacteria in the sediments of Little Round Lake relative to season are depicted in Fig. 2. Numbers of these bacterial agents of the sulfur cycle were significantly higher in the sediment than in the water column.

The population of Desulfovibrio in the sediment was lower in the autumn season than in March. Counts of 250 and 290 per gram were recorded for November, 1968 and October, 1969 respectively

...19

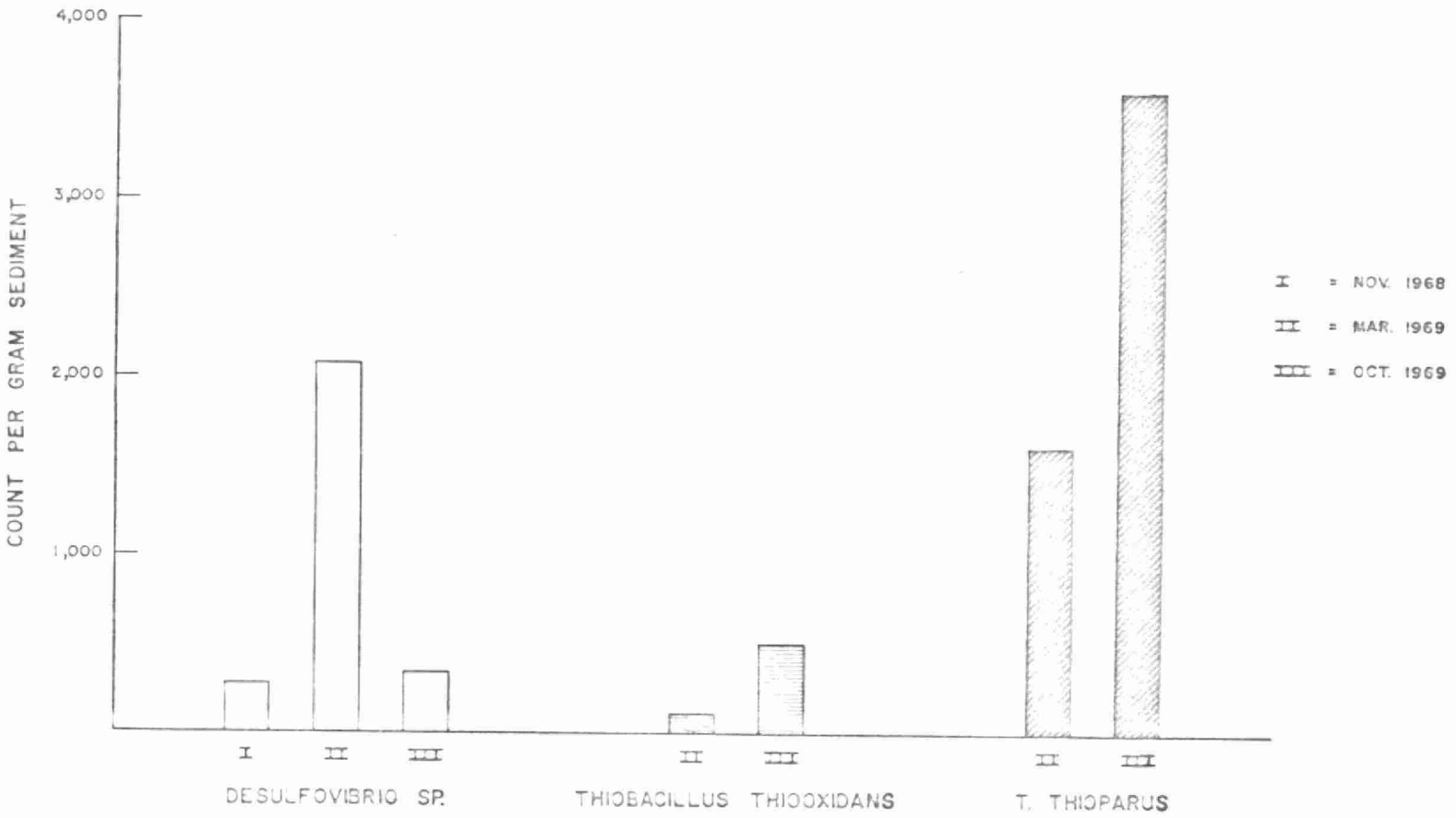

FIG. 2 — DISTRIBUTION OF SULFATE REDUCING AND AUTOTROPHIC SULFUR-OXIDIZING BACTERIA IN LAKE SEDIMENTS DURING SPRING AND AUTUMN

compared to 2100 per gram in March. The maximum count was somewhat lower than that reported by Bowers and Bishop (2) of 7.5×10^3 per gram sediment for Green Lake. These authors also found insignificant numbers of sulfate-reducers in the water samples.

The slow decomposition of organic matter deposited in the sediment during late summer and autumn may have released low M.W. organic compounds, e.g. organic acids which could possibly have stimulated growth of Desulfovibrio in the sediments over winter, accounting for higher populations in March than in October. Organic matter deposited in the sediment could result from the dead tissues of producers and consumers within the lake ecosystem and from wash-in of allochthonous organics from land after heavy rains. In the autumn of 1969, a tea-brown color was imparted to the water, presumably due to the latter factor.

Greater numbers of thiobacilli were found in the lake sediment in October than in March. T. thiooxidans was not detected in March, but 480 cells per gram sediment were found in October. Numbers of T. thioparus increased from 1400/g in March to 3400 in October. The sulfur bacteria which grew more abundantly in the lake water in summer and fall than in winter may have been carried down with particulate matter to the bottom resulting in a high population in the sediment during the autumn. Reduced populations of these bacteria in March may have been attributed to winter-kill by anaerobic conditions, H_2S toxicity, or other adverse environmental factors.

...21

Potaenko (12) observed a seasonal diversity in total bacterial numbers in lake water.

The distribution of aerobic and anaerobic heterotrophic bacteria in the water column of Little Round Lake in October, 1969, is illustrated in Fig. 3. Counts of aerobic bacteria were greater in the epilimnion than in the hypolimnion. The aerobic bacterial concentration was greatest in surface water, less at 5 m and least in the anaerobic zone from 10-16 m.

In surface water, aerobic bacteria growing on nutrient agar at 37 °C (11,000/100 ml) were more numerous than those bacteria showing growth on the same medium at 25 °C (6300) and 10 °C (4700). Decreased counts of aerobic heterotrophs at 5 m were noted, the count at 37 °C (2800) exceeding that observed for 10 °C (1500) and 25 °C (500). At 10, 12 and 14 meters in the lake profile, the numbers of bacteria growing on nutrient media at all 3 incubation temperatures were much lower (in the range of 200-400) than observed in epilimnetic waters.

Numbers of anaerobic heterotrophs grown at 25 °C on RCM were lower in the surface water, about the same level at 5 m, 12 m and 14 m and higher at 10 m than aerobic bacteria.

Hydrogen sulfide producing or sulfite-reducing clostridia grown on SPS media were less numerous than clostridia grown on RCM. The counts on SPS remained in the narrow range between 20-40/100 ml throughout the water column (Table II).

...22

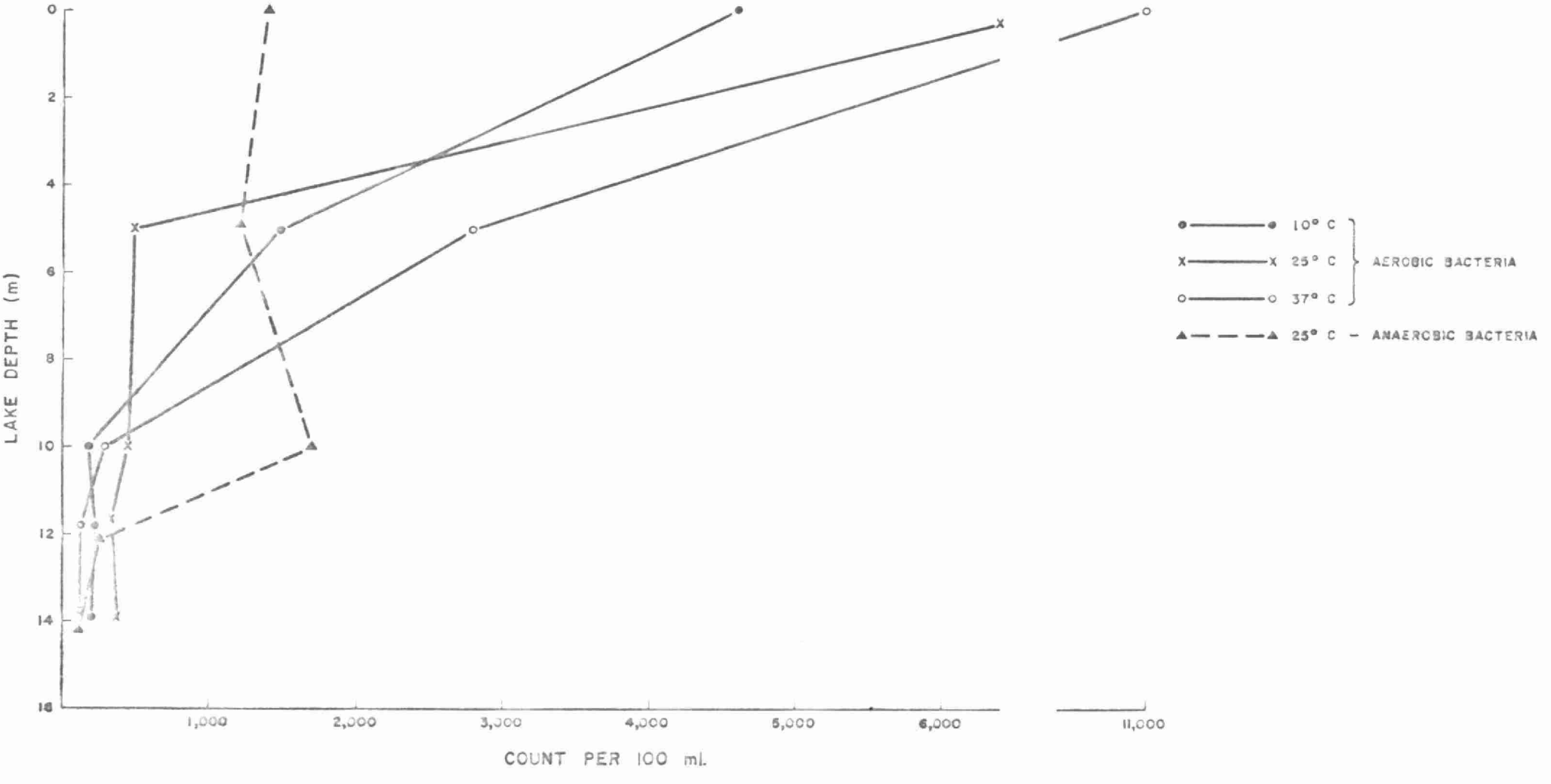

FIG. 3 – DISTRIBUTION OF HETEROTROPHIC BACTERIA IN WATER COLUMN ACCORDING TO GROWTH TEMPERATURES

Numbers of clostridia grown on RCM showed a constant distribution in the mixolimnion (about 1300/100 ml) and chemocline (1700) but decreased markedly in the H_2S zone to < 200/100 ml.

Higher counts of aerobic bacteria in the epilimnion may be attributed to a number of factors, among which the most important are the relative abundance of dissolved oxygen and organic matter in the upper strata during October. Chief sources or organic matter in this environment would be from the phytoplankton crop and from organics washed in from terrestrial sources. Reduction of bacterial numbers in the anaerobic zone due to H_2S toxicity must also be considered.

Bowers and Bishop (2) found no consistent pattern in the distribution of heterotrophic bacteria in Green Lake, although bacterial numbers were considerably higher in the mixolimnion than in the monimolimnion. These workers found many more gram negative than gram positive organisms at all depths, the majority of cells rod-shaped. Anaerobic and facultative anaerobic members of the Bacillaceae were isolated.

In a survey of English lakes, Taylor (16) found that total numbers of heterotrophic bacteria decreased with increasing depth in the epilimnion, but below the thermocline, the numbers were approximately the same at all depths. Growth of anaerobic bacteria was not stimulated by progressive depletion of dissolved oxygen in the hypolimnion during summer.

...24

In Little Round Lake, nitrifying bacteria were not detected in any water or sediment samples. Although very high ammonia concentrations were detected in the anaerobic zone, practically no nitrate or nitrite was found in this environment (8), suggesting that nitrification is not occurring in the lake. Anaerobic conditions would of course prevent growth of the obligate aerobic nitrifying bacteria. These autotrophs are quite sensitive to poisoning by H_2S and by many organic sulfur compounds. Certain toxic and/or nutritional factors may be involved in the inhibition of nitrification of the minute concentrations of ammonia found in the upper oxygenated layers. The ammonia concentration may even be below the minimum limit for growth, since these bacteria require NH_3 as their sole energy source; cells of Nitrosomonas may have been present in the water, although too widely dispersed to have been detected.

It should be indicated that only about 1 - 10% of the bacteria present in the aquatic environment can be detected by the enumeration of grown colonies on agar media (4).

The distribution of aerobic and anaerobic heterotrophic bacteria in sediment samples taken from Little Round Lake in October is depicted in Fig. 4. Psychrophilic types of aerobes predominated over mesophilics, while mesophilic anaerobes appeared to be more numerous than psychrophilic types in the sedimentary habitat. Aerobic bacteria growing at 10 °C on nutrient agar were about 3 and 2 times more numerous than those grown at 37 °C and 25 °C respectively.

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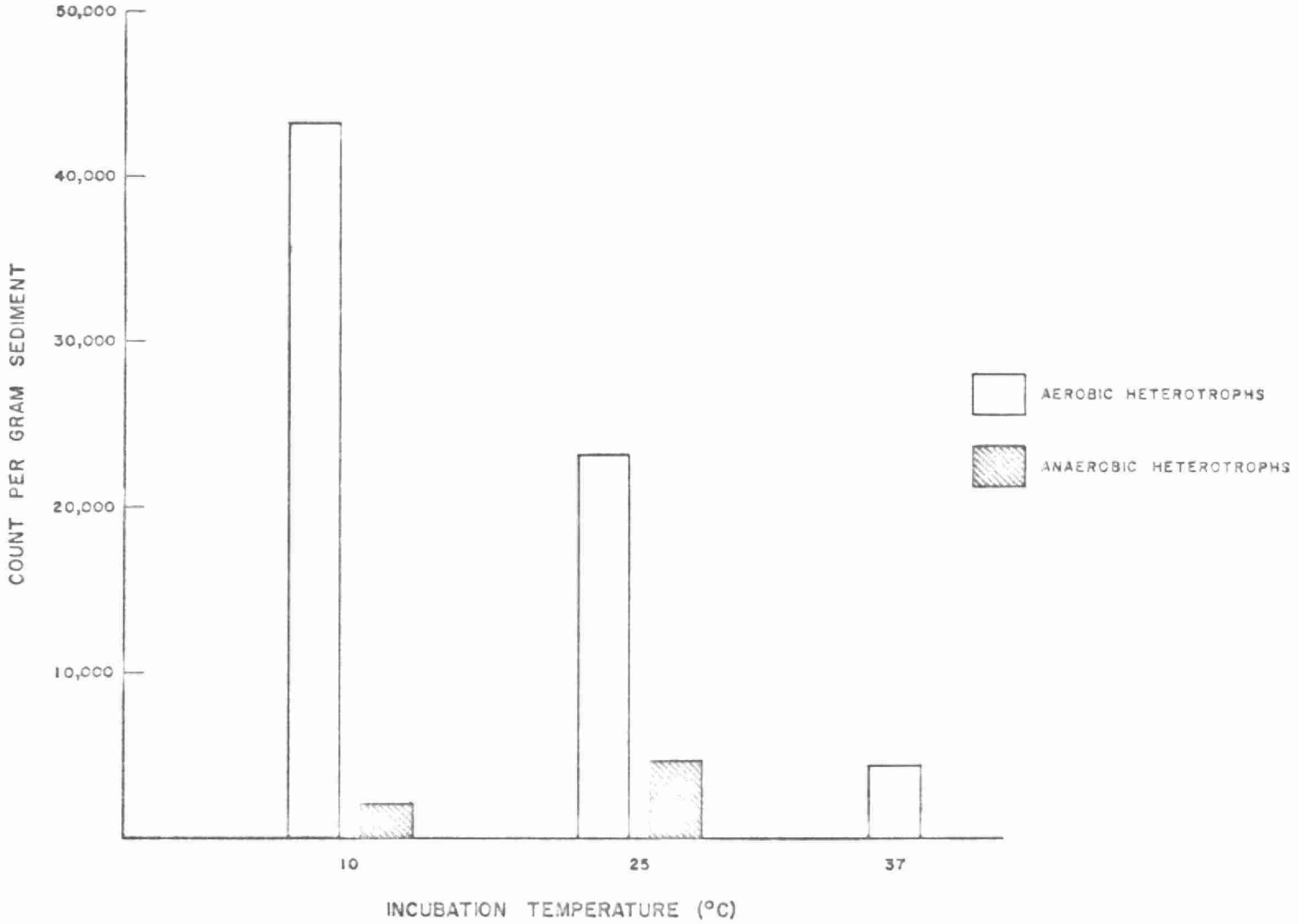

FIG. 4 — NUMBERS OF AEROBIC AND ANAEROBIC HETEROTROPHIC BACTERIA IN LAKE SEDIMENT ACCORDING TO OPTIMUM GROWTH TEMPERATURES.

Although numbers of clostridia growing at 25 °C on RCM were about three times as high as the count at 10 °C, anaerobic counts were considerably lower than aerobic counts.

Higher numbers of aerobic forms than anaerobic bacteria in the sediment at this time may have been attributed to the deposition of aerobic types associated with particulate organic matter from the upper water layers. Physical or nutritional growth requirements of some fastidious anaerobic microorganisms may have not been met, with the result that the count on RCM was not truly representative of the actual numbers of clostridia present. The majority of the bacteria growing on nutrient agar and RCM were gram positive spore-forming rods which may have been facultative anaerobes that showed preferential growth aerobically on nutrient agar.

In the water column, the relative proportion of aerobic heterotrophic bacteria growing on nutrient agar was approximately 20% gram positive bacilli to 80% gram negative rod forms.

In samples from the mixolimnion, pink colonies of a Rhodotorula sp. yeast appeared on nutrient agar with bacteria.

Colonies picked from anaerobic (RCM) plates indicated that a relative distribution of 40% gram positive spore-forming rods to 60% gram negative shorter rods occurred in the water samples.

This observation is not in agreement with many other workers including Taylor (17) who found that in aquatic habitats about 4% of the total bacterial population were gram positive and 96% gram negative,

...27

whereas in soil habitats, 43% of the total isolates were gram positive.

In the water of Little Round Lake at this season was a brownish color, possibly resulting from the wash-in of soil organic matter. It is possible that the high gram positive bacterial population in the water and sediment samples may have some relationship with soil (terrestrial) sources.

In the sediment samples about 90% of the bacteria grown aerobically on nutrient agar were gram positive rods (with or without spores), and 10% gram negative asporogenous rods of different sizes. Of the bacteria grown aerobically on RCM, about 70% were gram positive spore-formers and 30% gram negative rods (with or without spores).

Two species of Clostridium which reduced sulfate were isolated from the sediment. One organism characterized by gram positive cigar-shaped cells with terminal clubbed endospores was identified as Cl. pectinovorum. The other large gram negative anaerobic sporulating rod was not identified. Both isolates produced H_2S from the reduction of sulfate and from the degradation of proteose-peptone.

The relatively high populations of members of the Bacillaceae in Little Round Lake presents an unusual situation for a fresh water lake. Environmental factors responsible for this phenomenon are unknown at the present time.

From the preliminary results of the bacteriological survey of Little Round Lake, it can be concluded that more definitive studies should be carried out on the characterization and taxonomy of

...28

heterotrophic bacteria, and on the relative distribution and activities of sulfur bacteria in this meromictic lake to gain more knowledge of the microbiological processes involved in this ecosystem.

- SUMMARY -

1. A bacteriological study of Little Round Lake, Ontario, revealed that populations of chemosynthetic sulfur bacteria were higher in the water column and sediment in the fall season than in spring. Thiobacillus thioparus was predominant over T.thiooxidans in the lake. In the summer and autumn, maximum numbers of thionic bacteria appeared at 2 m in the epilimnion and at 10-12 m in the chemocline.
2. Numbers of sulfate-reducers were very low (< 50/100 ml) or were not detected in the water column at all seasons. In the sediment, populations of Desulfovibrio were significantly higher in March than in October.
3. Nitrifying bacteria were not detected in water or sediment.
4. Higher counts were obtained for aerobic heterotrophic bacteria than anaerobes. Counts of aerobic bacteria decreased with increasing depth in the epilimnion and fell to a constant low level in the H_2S zone below 11 m. Numbers of anaerobic bacteria

...29

were relatively constant with depth in the epilimnion but declined markedly in the H_2S zone.

5. In the water column and especially the sediment, the relatively high proportion of gram positive rods (Bacillaceae) to gram negative rods appeared peculiar for an aquatic environment. Rod-shaped cells were the predominant forms in the lake environment.

- Acknowledgements -

The author's appreciation is expressed to Dr. R.S. Brown and Mr. R. McNeely, Biology Department, Queen's University, for their co-operation in bacteriological sampling and discussions regarding the ecology of Little Round Lake, Thanks are also due to Mr. Tibor Lovasz, Bacteriology Branch, for technical assistance with the bacterial analyses.

...30

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